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Insect Repellent/Antifeedant Activity of 2,4-Methanoproline and Derivatives against a Leaf- and Seed-Feeding Pest Insect

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2,4-Methanoproline is a natural product isolated from the seeds of *Ateleia herbert smithii* Pittier that was formerly suggested to have insect repellent/antifeedant activity; however, this was not tested quantitatively. In this study the insect repellent/antifeedant potency of methanoproline was measured against larvae of the cotton leafworm, *Spodoptera littoralis* (Boisd.), and adults of the cowpea weevil, *Callosobruchus maculatus* (F.). In addition, several N-alkyl, amino, and nitrile derivatives of methanoproline with varying stereodemanding substituents were synthesized and also tested. It was shown that in *S. littoralis* methanoproline itself did not show any significant activity but that derivatives **5**, **7**, **8**, and **10** did show a reasonable repulsive/antifeedant activity that was comparable to the commercial repellent DEET. A significant repellent activity was scored for methanoproline in adults of *C. maculatus* that was similar to DEET.

KEYWORDS: 2,4-Methanoproline; *Ateleia herbert smithii*; insect repellent; *Spodoptera littoralis*; *Callosobruchus maculatus*

INTRODUCTION

It is estimated that almost 30% of planted crops is lost due to attack of feeding insects, wastage, or disease (*1*). Therefore, there is a continuous drive to develop new compounds with an insect repellent activity in order to protect the food and feed reserves. Applying the active repellent compounds around the storage areas keeps the pest insects at a distance, thus protecting the reserves from their attack. Nowadays, more and more attention is paid to the use of natural products because of selectivity reasons and environmental problems related to persistent residues.

Azadirachtin, isolated from the neem tree *Azadirachta indica*, and Angulatin A, from the bark of *Celastrus angulatus Max.*, are such natural products with good insect repellent features. However, azadirachtin and angulatin A are complex molecules which only make the isolation from natural sources feasible for their use.

In some initial publications on the isolation of new derivatives from the seeds of *Ateleia herbert smithii* Pittier, growing at the coasts of Puerto Rico, it was suggested that 2,4-methanoproline would be responsible for the observed insect repellent activity of these seeds (*2*−*5*). 2,4-Methanoproline and related nonprotein amino acids are also present in legumes of the genus *Bocoa* (*6*). Therefore, methanoproline rose to our attention in order to develop a general synthesis, also because of its very strained 2-azabicyclo[2.1.1]hexane skeleton which is quite unique in nature. During our work further interest in 2-azabicyclo[2.1.1]-hexanes originated in view of the construction of epibatidine analogues in the search for high affinity and high subtype selectivity at the nicotinic acetylcholine receptor (nAChR) (*7*).

Although some interesting synthetic routes toward 2,4-methanoproline have been described (*2*−*5*), no routes to its derivatives are available nor has there been any real quantitative proof of the insect repellent/antifeedant properties to date. The formerly described methods are not always suitable for large-scale synthesis of methanoproline. Therefore, it was decided to develop new synthetic methods that allow larger production of the compound and synthesis of several derivatives of methanoproline. The potential as an insect repellent was compared with the commercial repellent DEET (*N*,*N*-diethyl-3-toluamide) (*8*, *9*).

MATERIAL AND METHODS

Synthesis. In the context of this study, several methods have been developed to produce the 2-azabicyclo[2.1.1]hexane skeleton (*10*), 2,4-methanoproline (*11*), and its derivatives (*12*). Details on the synthesis of these compounds as well as the physical and spectral data are presented in ref *12* and in the corresponding on-line Supporting Information.

The preferential method for production of 2,4-methanoproline is the hydantoine route, whereas the preferred method for production of the cyano and aminomethyl derivatives is the addition−intramolecular substitution sequence. Using these methods, methanoproline **1** and methanoproline derivatives **2**−**14** were prepared in pure form for the in vivo testing of the insect repellent/antifeedant activity. The evaluated

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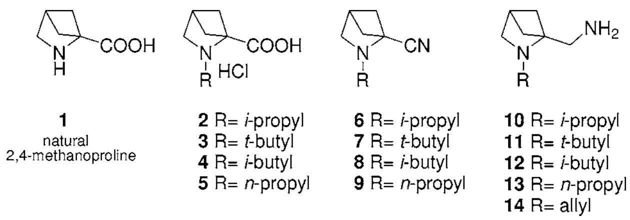
Scheme 1. Tested Methanoproline Derivatives



derivatives are shown in **Scheme 1**. The synthesized derivatives were all characterized by spectroscopic means using ^{1}H and ^{13}C NMR, IR, and MS.

Insect Bioassays. In a first series of experiments, pure compounds were tested for insect repellent/antifeedant activity against larvae of the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). This is a major herbivorous pest insect in cotton, vegetables, and ornamentals in Europe, the Mediterranean region, and northern America, and levels of resistance to classical insecticides and novel insecticide groups have already been reported (*13*). For these experiments young (0−2 day old) larvae in the last (sixth) stage were selected from a continuous culture at the Laboratory of Agrozoology of Ghent University, which were kept at standard conditions of 23 ± 2 °C, 65 ± 5% RH, and a 16:8 (L:D) photoperiod as described previously (*14*, *15*).

In the screening bioassay with *S. littoralis* a 0.1% solution of all compounds was made in distilled water (compounds **1**−**5**) or acetone (compounds **6**−**14**). Freshly cut castor bean leaves, *Ricinus communis* L., were sprayed uniformly on the upper side with 4 mL of solution in a standardized Cornelis' potter tower (*16*). As control, leaves were treated with distilled water or acetone alone. After solvent evaporation in a fume hood, leaves were placed in a 35 × 23 × 12 cm plastic container with the treated leaf on one side of the container and the control leave on the other side. Then 30 last-instar *S. littoralis* larvae were placed in the central area of the container without making contact with the leaves. The number of larvae on each castor bean leaf was scored after 1 day of treatment. Data are expressed as means ± SEM per compound based on a minimum of 2−4 replicates. Fifty percent of larvae on the control and the treated leaf represents a homogeneous distribution over the two leaves (which was the case in the control treatments); 0% larvae on the treated leaf indicates a complete insect repellent action.

Next to methanoproline and its derivatives, the insect repellent activity of DEET (*N*,*N*-diethyl-3-toluamide; Sigma Co., St-Louis, MO) that is being used as a commercial insect repellent in several applications was tested with *S. littoralis*. In the case of compound **8,** which possessed a high repellent activity, a range of lower concentrations of this derivative was tested and data subjected to nonlinear regression analysis to calculate the RC_{50}, the concentration that causes 50% of repellence in treated insects (*14*, *15*).

In a third series of experiments the insect repellent activity of 2,4-methanoproline **1** itself and compounds **5**, **7,** and **10** was tested against the cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). The selection of compounds was based on the scored repellent activity from the previous screening assay together with the fact that compound **5** was selected from the *N*-alkyl 2,4-methanoproline derivatives, compound **7** from the nitrile derivatives, and compound **10** from the amine derivatives. This *C. maculatus* is one of the most important pest insects of stored grains of legumes such as peas and beans. These bruchid weevils attack the seeds during storage, severely affecting the quality and storability of the produce. In severe periods of infestation postharvest seed losses caused by *C. maculatus* can reach 100% within a period of 6 months. In addition, high resistance to classical and more recent insecticide groups has already been reported (*13*). For the experiments adults were selected from a continuous culture at the Laboratory of Agrozoology of Ghent University, which was kept at standard conditions of 28 ± 2 °C, 65 ± 5% RH, and a 16:8 (L:D) photoperiod (*14*, *15*, *17*)

In this bioassay with C. maculatus 2,4-methanoproline **1** and compounds **5**, **7,** and **10** were prepared as a 0.1% solution in distilled water. Forty chick peas (*Cicer arietinum* L.) were dipped uniformly for 10 s in 10 mL of solution (*17*). As control, chick peas were treated with distilled water alone. After solvent evaporation in a fume hood, chick peas were placed in a 17 × 11 × 7 cm plastic container with a group of treated peas on one side of the container and a group of control peas on the other. Then 40 adults of *C. maculatus* were placed in the central area of the container without making contact with the peas. The number of eggs dumped on each pea was scored after 1 day of treatment. Data are expressed as means ± SEM per compound based on a minimum of two replicates. Fifty percent of eggs on the control and treated pea group represents a homogeneous distribution over the two groups (which was the case in the control treatments); 0% eggs on the treated peas indicates a complete insect repellent action (*14*, *15*, *17*).

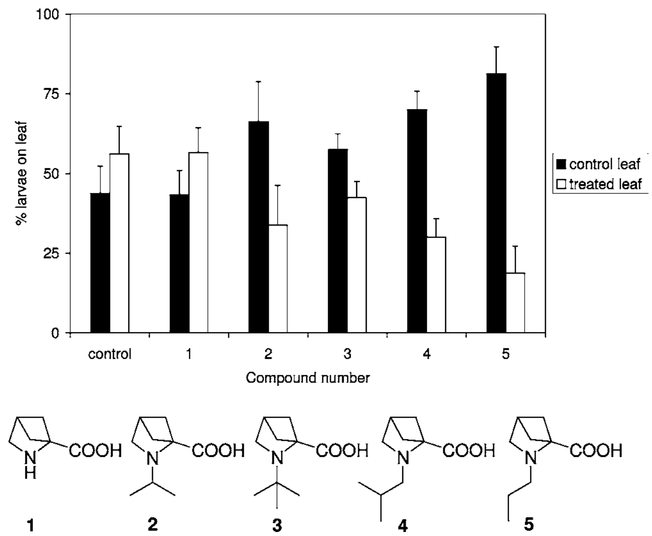


Figure 1. Distribution of the larvae of *S. littoralis* on control and treated leaf (4 mL of a 0.1% solution in distilled water).

RESULTS AND DISCUSSION

The data on insect repellent/antifeedant activity against *S. littoralis* for 2,4-methanoproline **1** and the *N*-alkyl 2,4-methanoproline derivatives **2**−**5** are presented in **Figure 1**. It is noteworthy to see that here 2,4-methanoproline **1** did not show insect repellent activity, although such activity has been suggested several times in the literature. However, it should be remarked that the activity was tested only in *S. littoralis* as a test insect; therefore, it cannot be excluded that 2,4-methanoproline has no repellent activity at all. On the other hand, a significant repellent/antifeedant activity was observed for the *N*-propyl derivative **5**.

The next series of tests was performed on the corresponding nitrile derivatives **6**−**9**. From these results, as presented in **Figure 2**, it can be concluded that not all the nitrile derivatives possess an insect repellent activity. The *N-tert*-butyl **7** and *N*-isobutyl **8** derivatives showed a significant activity, but the *N*-isopropyl **6** and *N*-propyl **9** derivatives are almost inactive toward the tested larvae.

A typical view of the tests, e.g., for compound **8**, is shown in **Figure 3a** and **b**. **Figure 3a** shows the situation shortly after the start of the experiment with a predominance of larvae on the nontreated leaf on the right side of the container. **Figure 3b** shows the situation after 6 h from which it is clear that considerably more damage has occurred to the nontreated leaf (right) in comparison with the leaf treated with a solution of the methanoproline derivative **8** (left).

In the third series of tests with *S. littoralis* the amines **10**−**14** were tested, and again large differences in activity were found

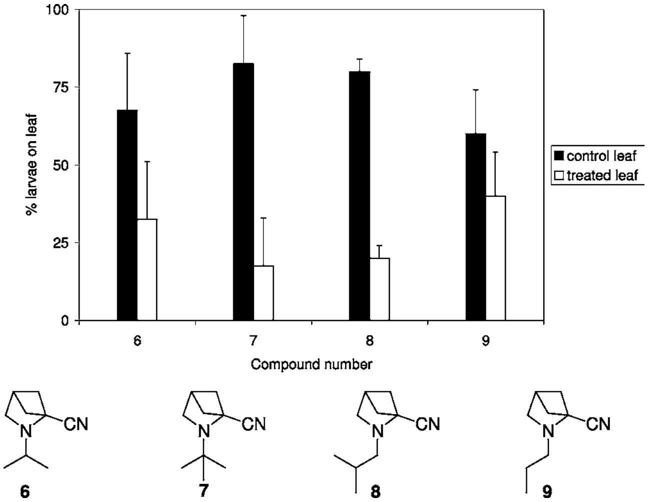


Figure 2. Distribution of the larvae on control and treated leaf (4 mL of a 0.1% solution in acetone).

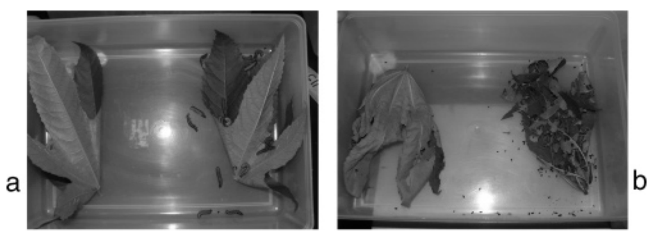


Figure 3.

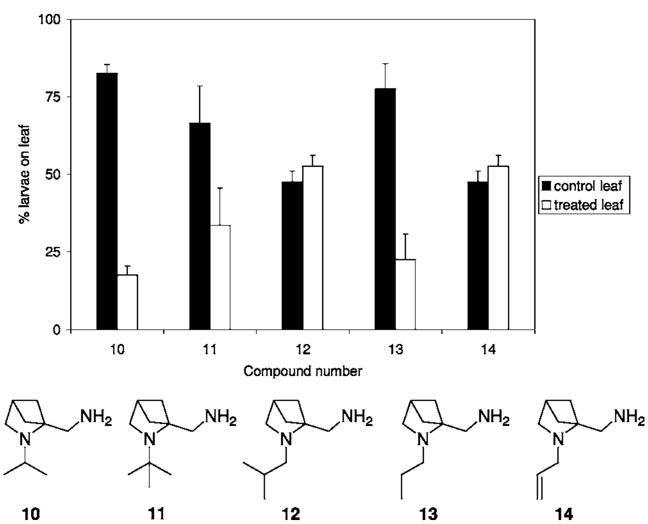


Figure 4. Distribution of the larvae of *S. littoralis* on control and treated leaf (4 mL of a 0.1% solution in acetone).

depending on the *N*-alkyl substituent group (**Figure 4**). It was of interest that, in particular, the *N*-isopropyl derivative **10** showed a potent activity.

Although methanoproline did not show any significant activity on the tested larvae, which was much to our surprise, it was observed that four other compounds showed a considerable insect/antifeedant repellent activity against larvae of *S. littoralis*. The compounds **5**, **7**, **8,** and **10** gave a result of 80% preference for the untreated leaves in the experiment described with larvae of *S. littoralis*.

The results obtained with methanoproline and derivatives **2**−**12** are not obvious to interpret in terms of the structure−activity relation since activity was detected for a different functional group (CN, COOH, and CH_2NH_2) and for different alkyl substituents (R = propyl, isobutyl, *tert*-butyl, and isopropyl). The 2-azabicylo[2.1.1]hexane is presumably not the only required structural moiety to induce activity; otherwise, all the tested compounds should show some activity. Therefore, a synergistic effect between the skeleton, functional group, and side chain needs to be important for activity. Further experiments with other pest insects will have to be performed in order to get a good view of the repellent/antifeedant potency of the 2-azabicyclo[2.1.1]hexanes.

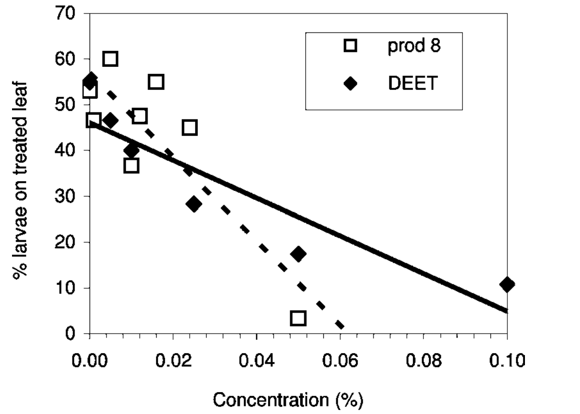


Figure 5. Insect repellent activity of different concentrations of derivative **8** and of the commercial repellent DEET against larvae of *S. littoralis.*

Further, a concentration−response bioassay with product **8** was performed based on its high repellent activity in the first screening bioassay. Different concentrations were made in acetone ranging from 0.001% to 0.1%, and the insect repellent activity was tested as described above. Then an RC_{50} causing 50% repellency was estimated by nonlinear regression analysis and compared to that for the commercially used repellent DEET. From **Figure 5** it was clear that the concentration−response curves of derivative **8** and DEET nearly overlapped. A respective RC_{50} concentration of 0.0248% and 0.0356% could be calculated. This RC_{50} caused only 25% of the larvae to be present on the treated leaf, representing 50% repellency.

In summary, 2,4-methanoproline and several related 2-azabicylo[2.1.1]hexanes have been tested for their insect repellent/antifeedant activity toward larvae of *S. littoralis.* Although it was previously suggested that 2,4-methanoproline had insect repellent properties, the biological testing showed no significant repellent activity. However, a significant repellent activity, which was comparable to the activity of the commercially available DEET, was found for some of the related derivatives.

In a final experiment the repellent activity of 2,4-methanoproline **1**, the *N*-alkyl derivative **5**, the nitrile derivative **7,** and the amine derivative **10** was tested against adults of *C. maculatus*. As shown in **Figure 6** it was clear that methanoproline exerted a significant insect repellent action of 80% against cowpea weevils to dump eggs on treated peas. It is of interest that this repellent potency is similar to that of the commercial repellent DEET at a similar concentration of 0.1%. The other three derivates tested also showed a potent activity resulting in 60−70% preference for the untreated peas.

On the basis of all the obtained results it is suggested that the four compounds **5**, **7**, **8,** and **10** possess a high insect repellent/antifeedant activity with a realistic potency for use in practice. For 2,4-methanoproline itself the repellent potency is suggested to be more insect species dependent, but the strong activity in *C. maculatus* is promising for practical use. However, further research is needed to address the question of the

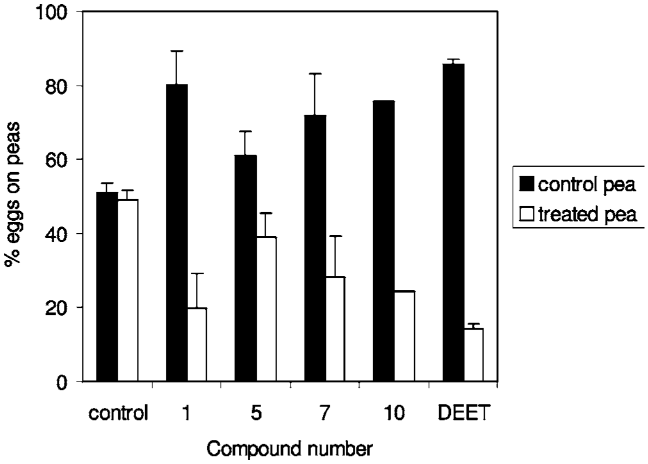


Figure 6. Distribution of the eggs of *C. maculatus* on control and treated peas (with methanoproline **1** and derivatives **5**, **7,** and **10** at 0.1% in water).

mechanism of the interaction of methanoproline and its derivatives with insect larvae and adults.

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